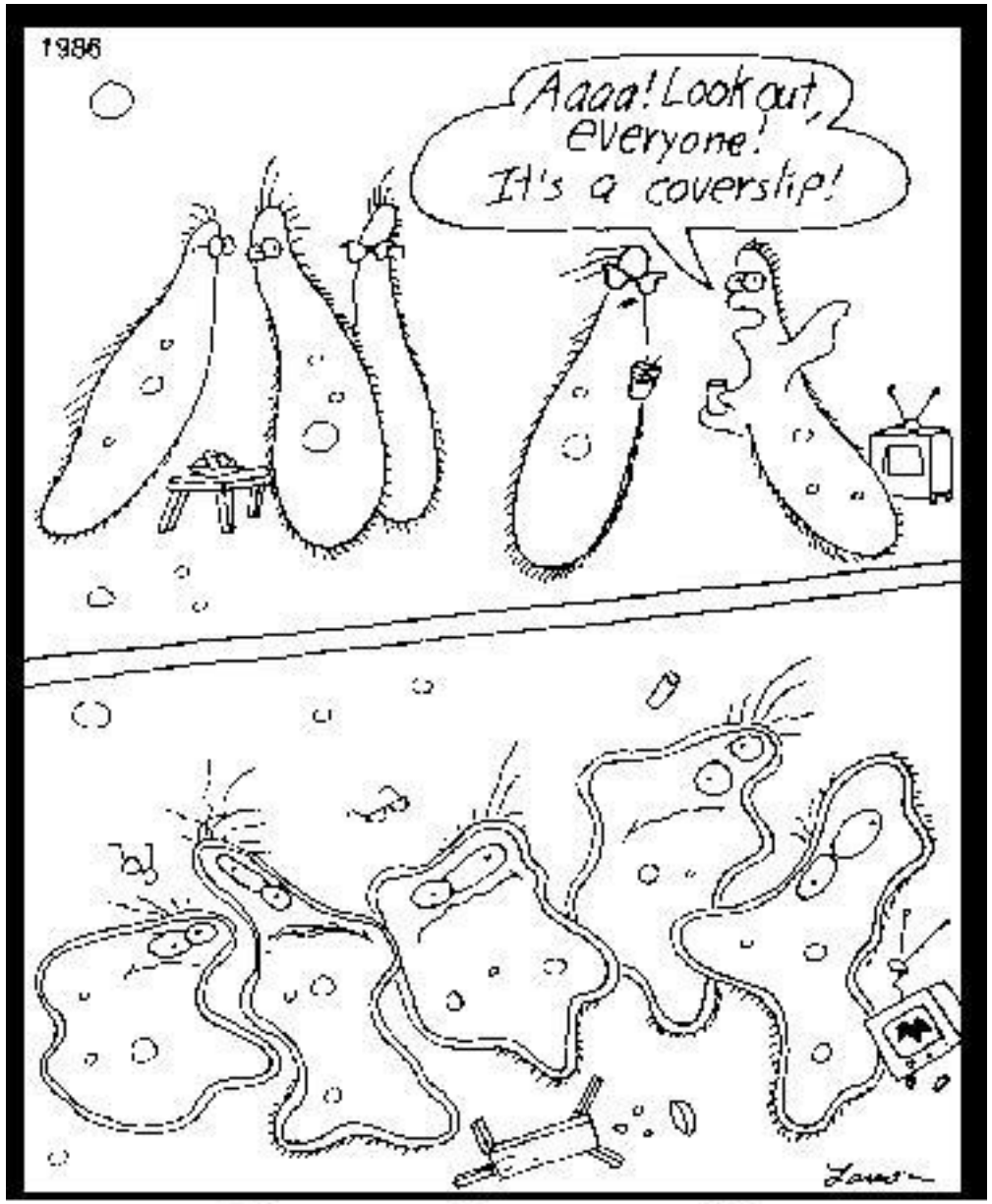
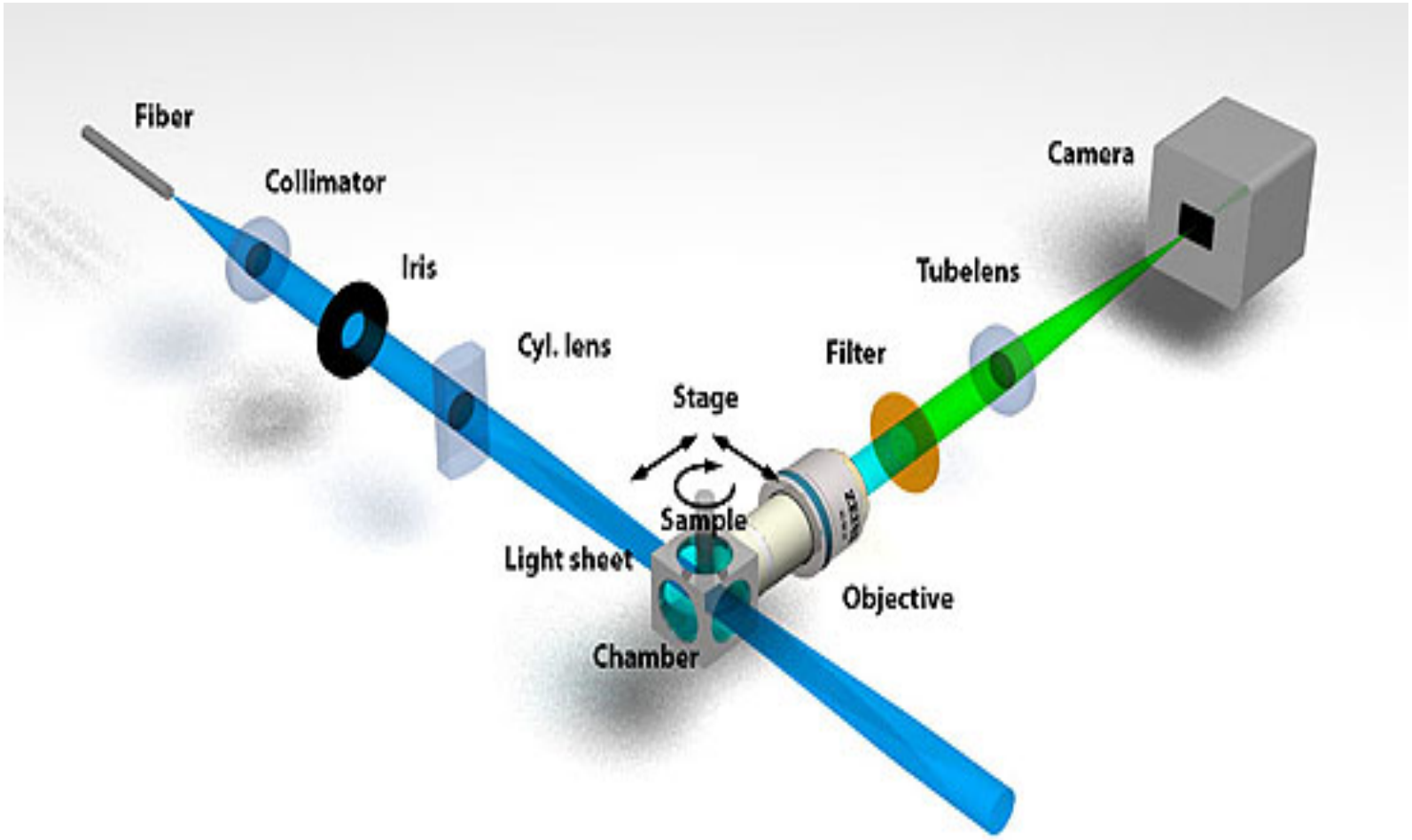
Selective plane illumination microscopy – SPIM (light sheet microscopy)



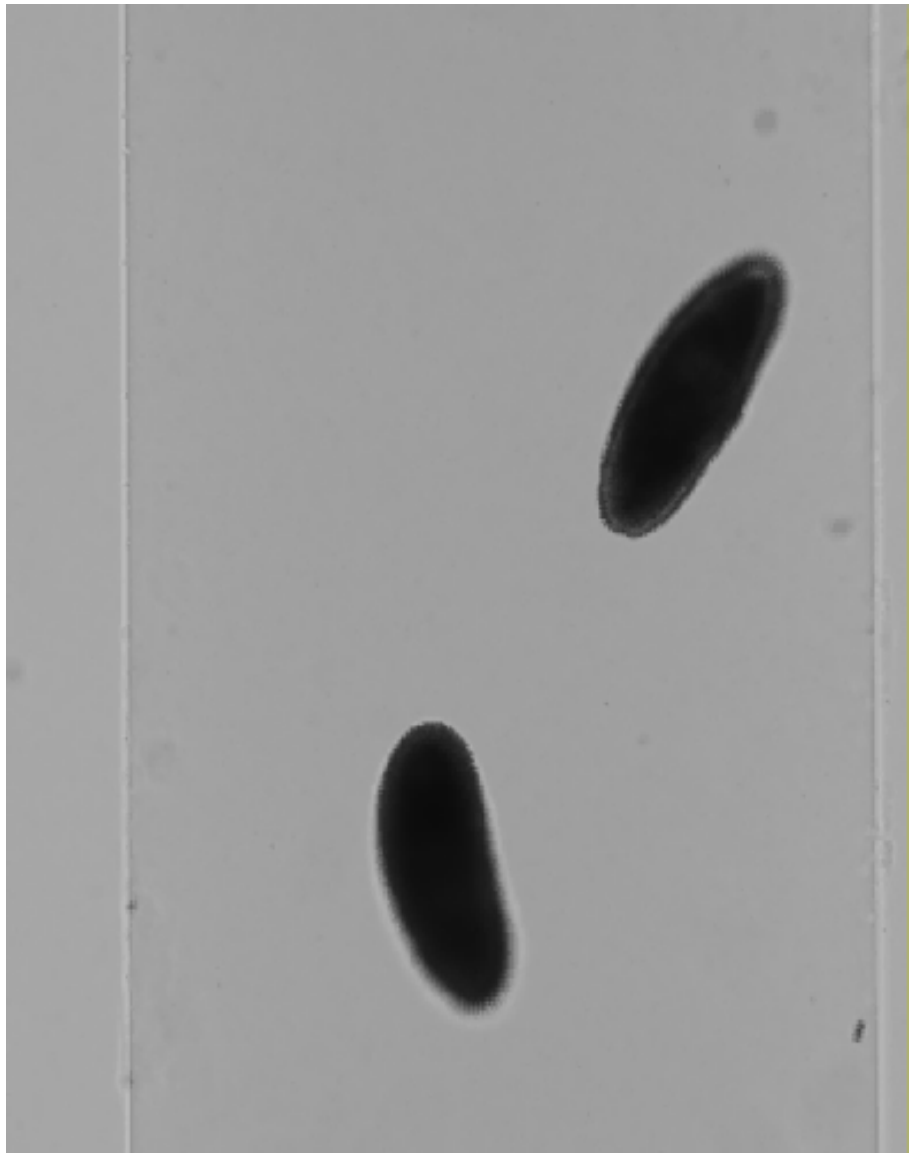
Life on a microscope slide

Sticking biology to flat glass is not very physiological...

SPIM



http://www.huisken.org/jan/spim.html



Pavel Tomancak

Selective plane illumination microscopy - SPIM

Field illuminating technique (detector: CCD, EM CCD, sCMOS camera)

Temporal resolution typically tens to hundreds of ms/image in “standard” FOV – 512x512 pixels

Multiple angle image acquisition – isotropic resolution x,y,z

Low photobleaching

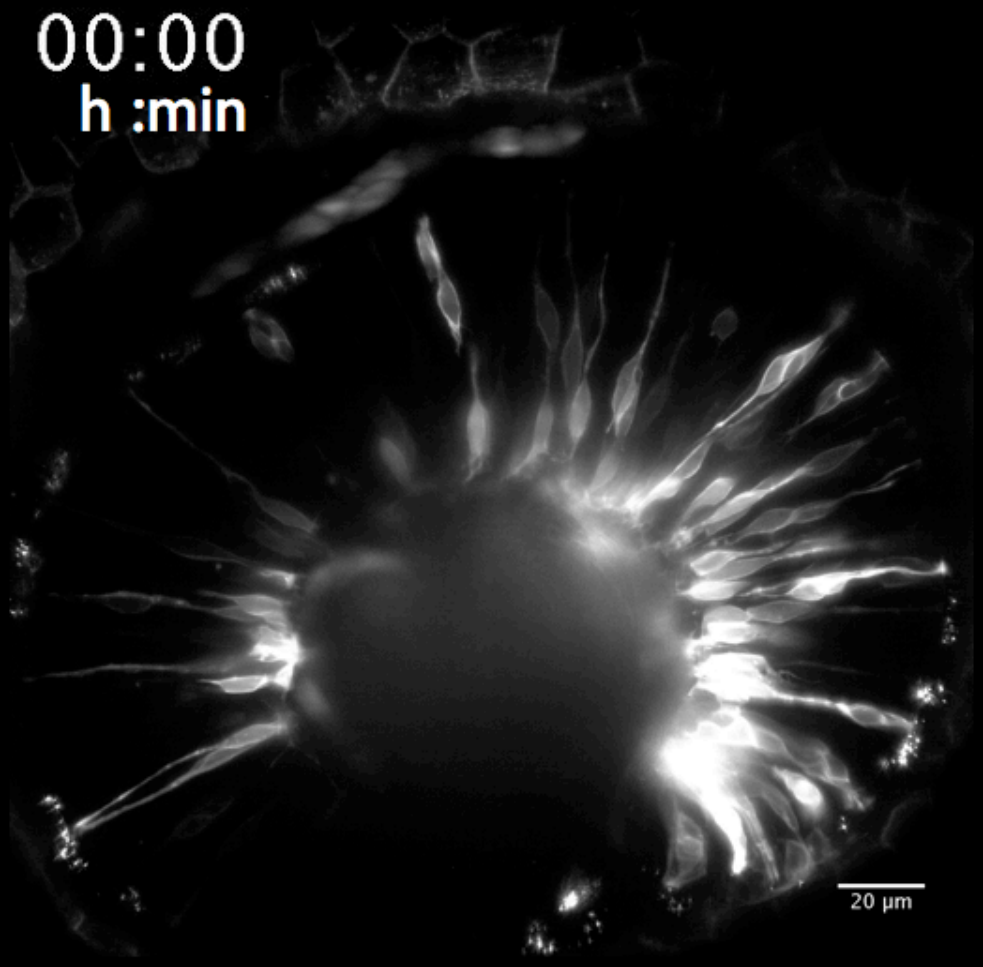
Compromise between size of FOV and resolution

SPIM – what is it good for ?

- live cell imaging of embryos

- multiple angle imaging of fixed and living specimens

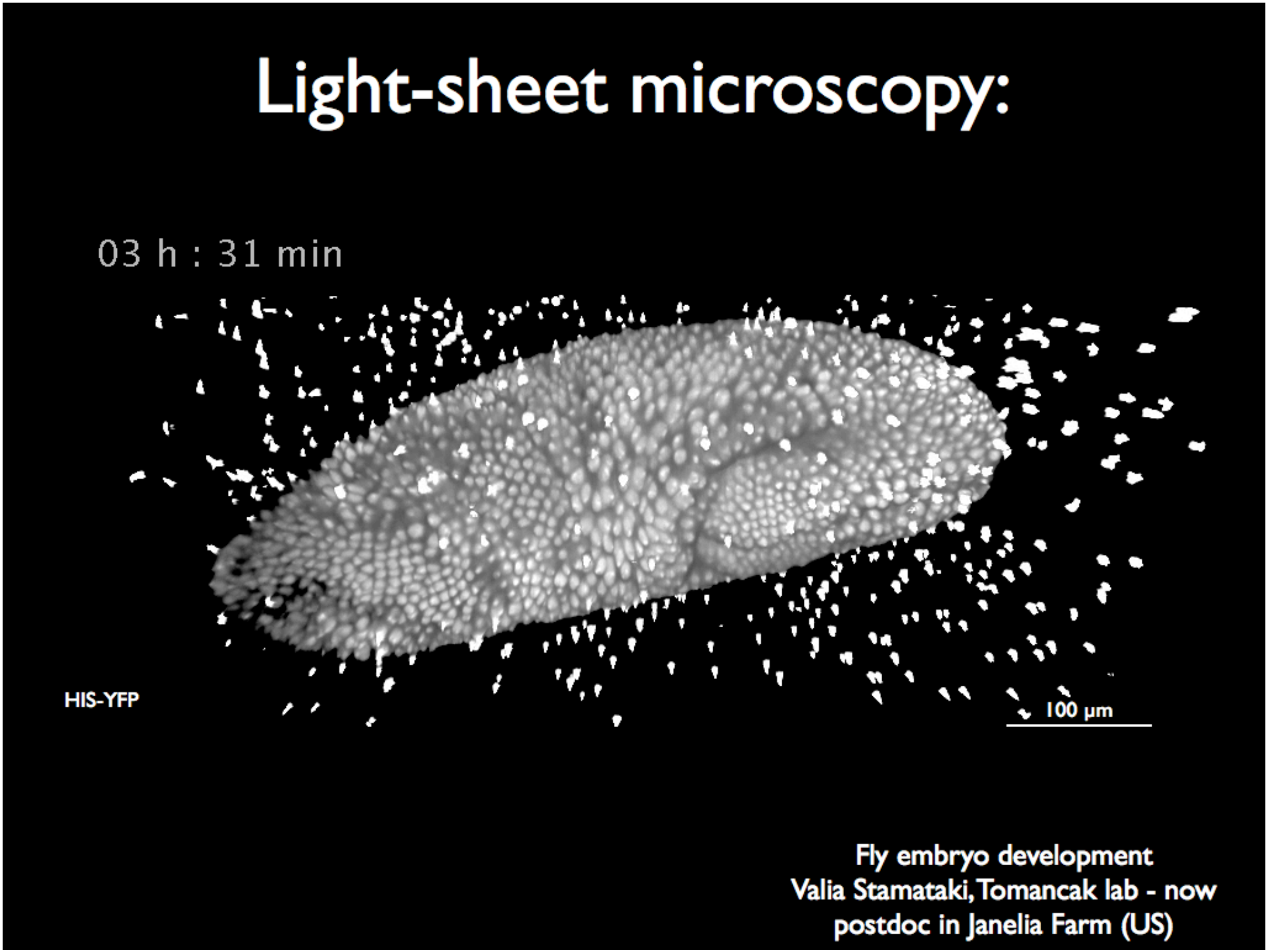
Neurogenesis in *Danio rerio* retina:



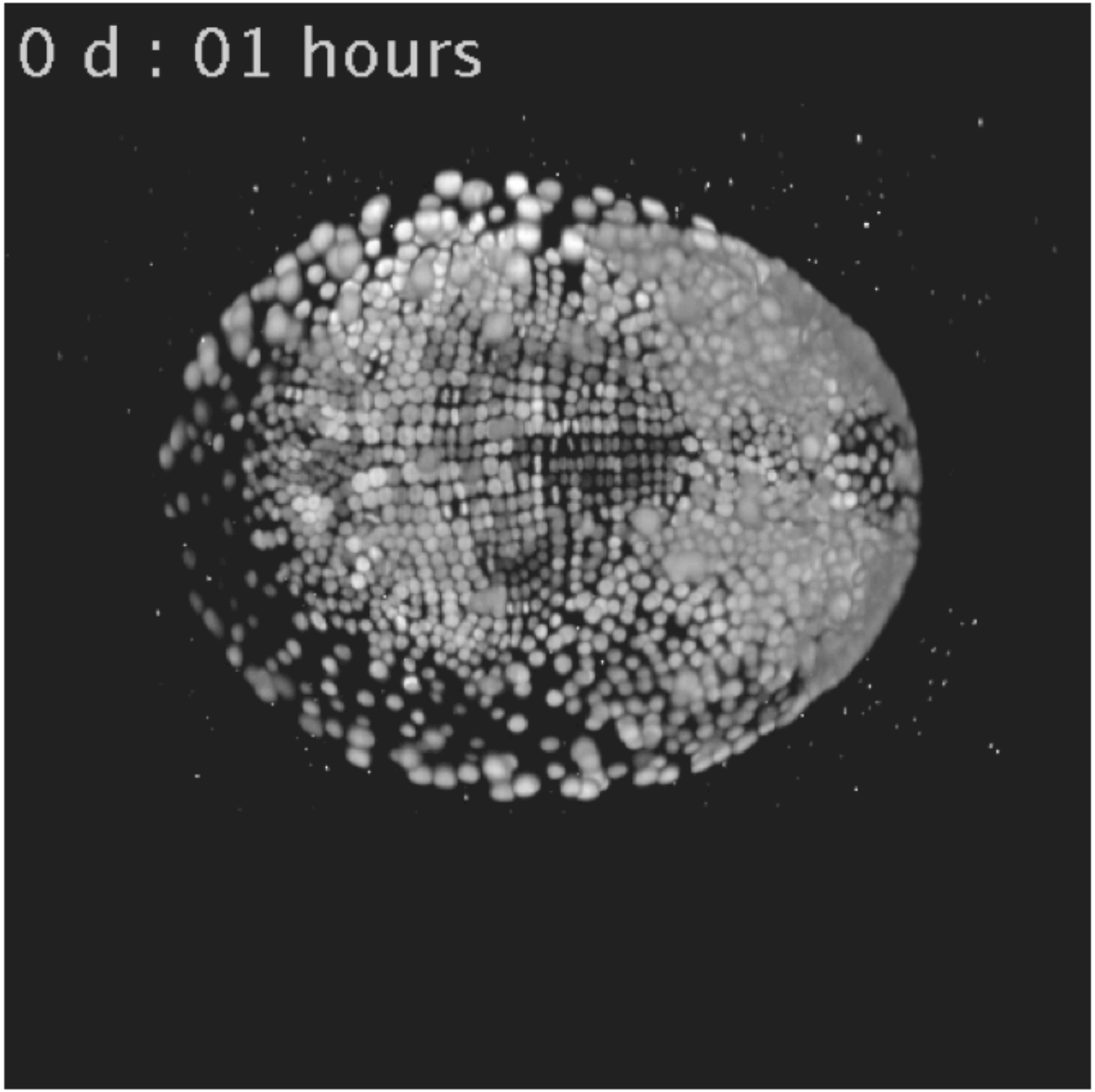
Ath5-RFP

Jaroslav Icha, Norden Lab, MPI-CBG

Light-sheet microscopy:

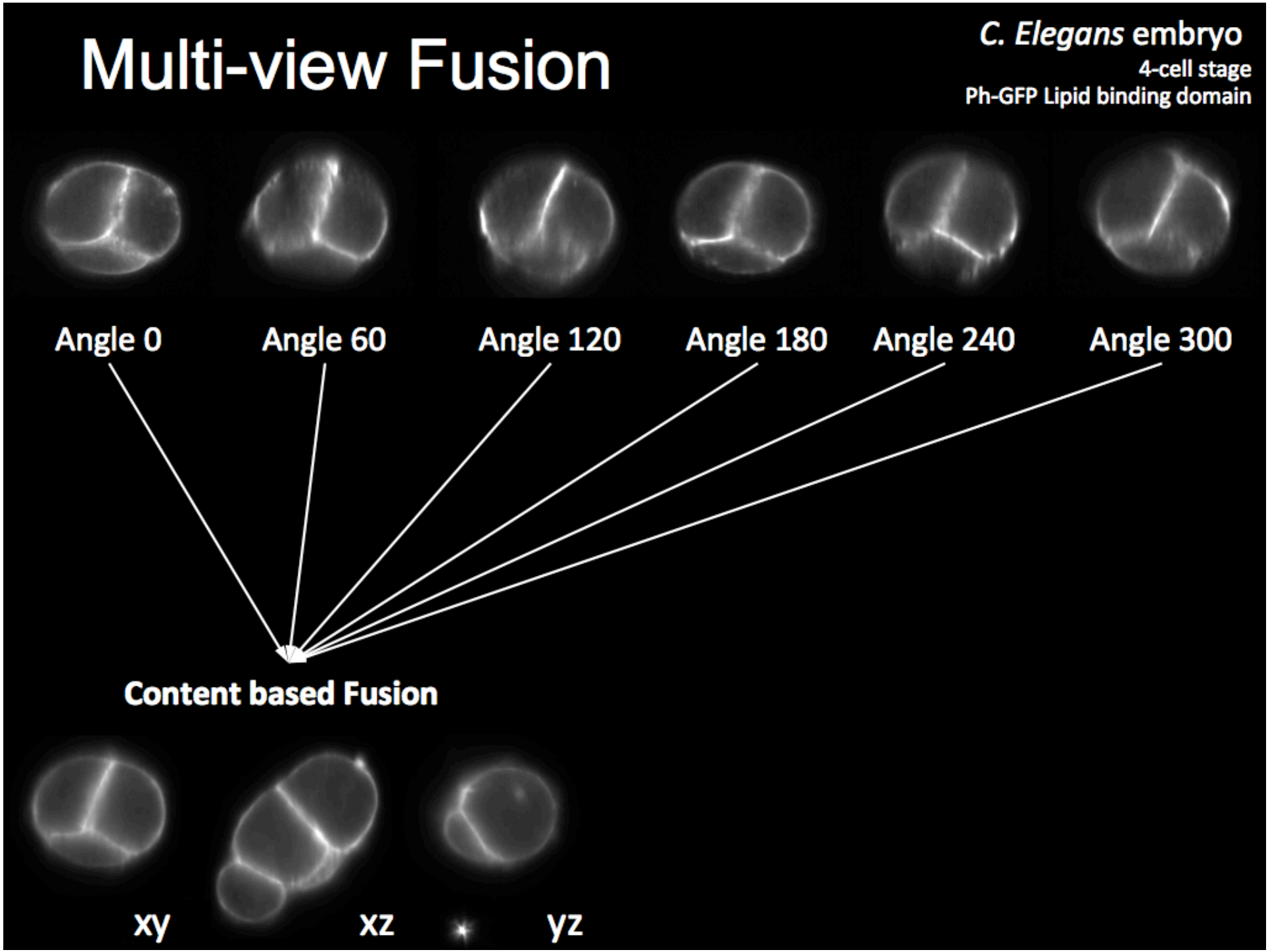


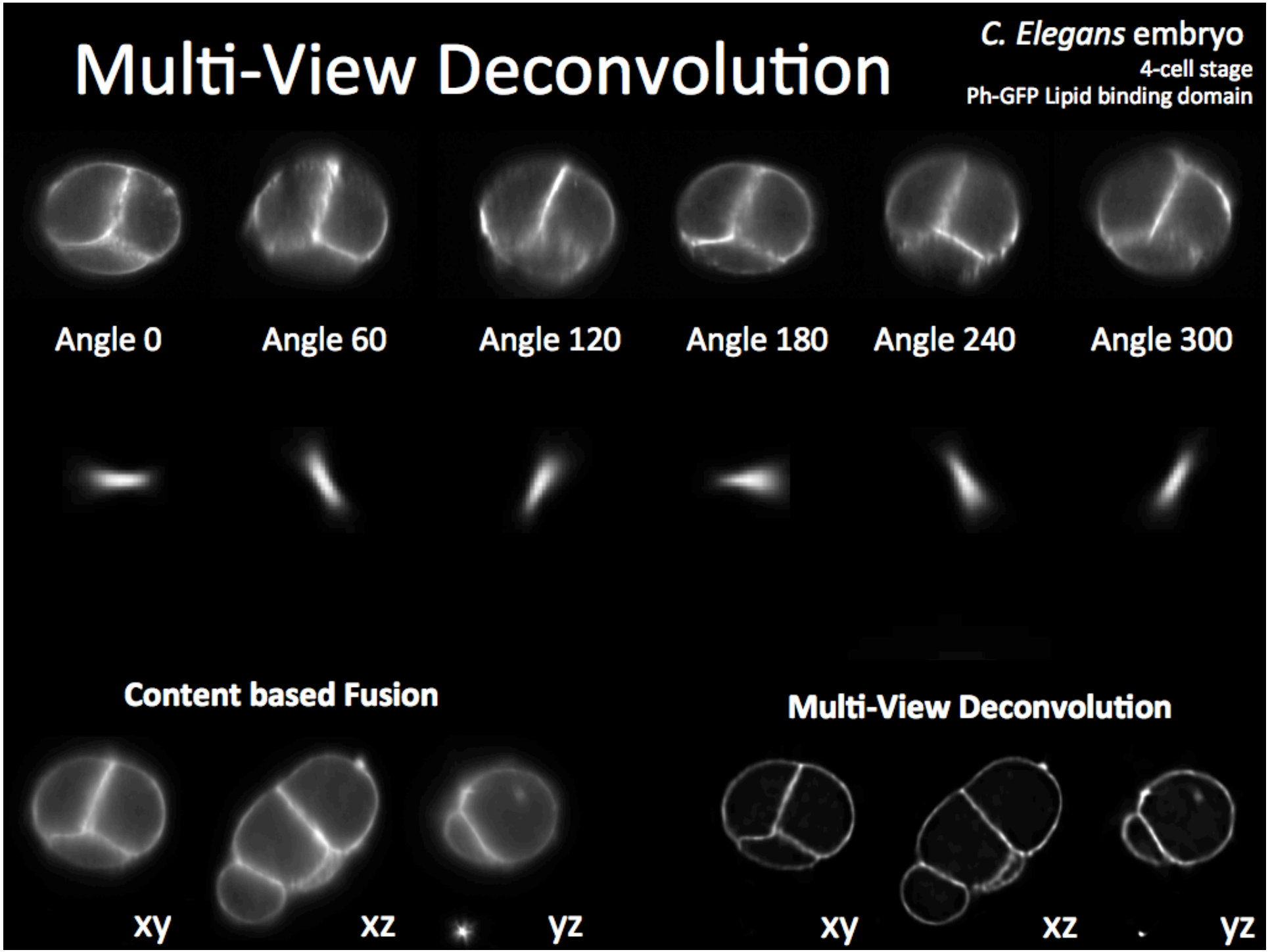
Fly embryo development
Valia Stamataki, Tomancak lab - now
postdoc in Janelia Farm (US)



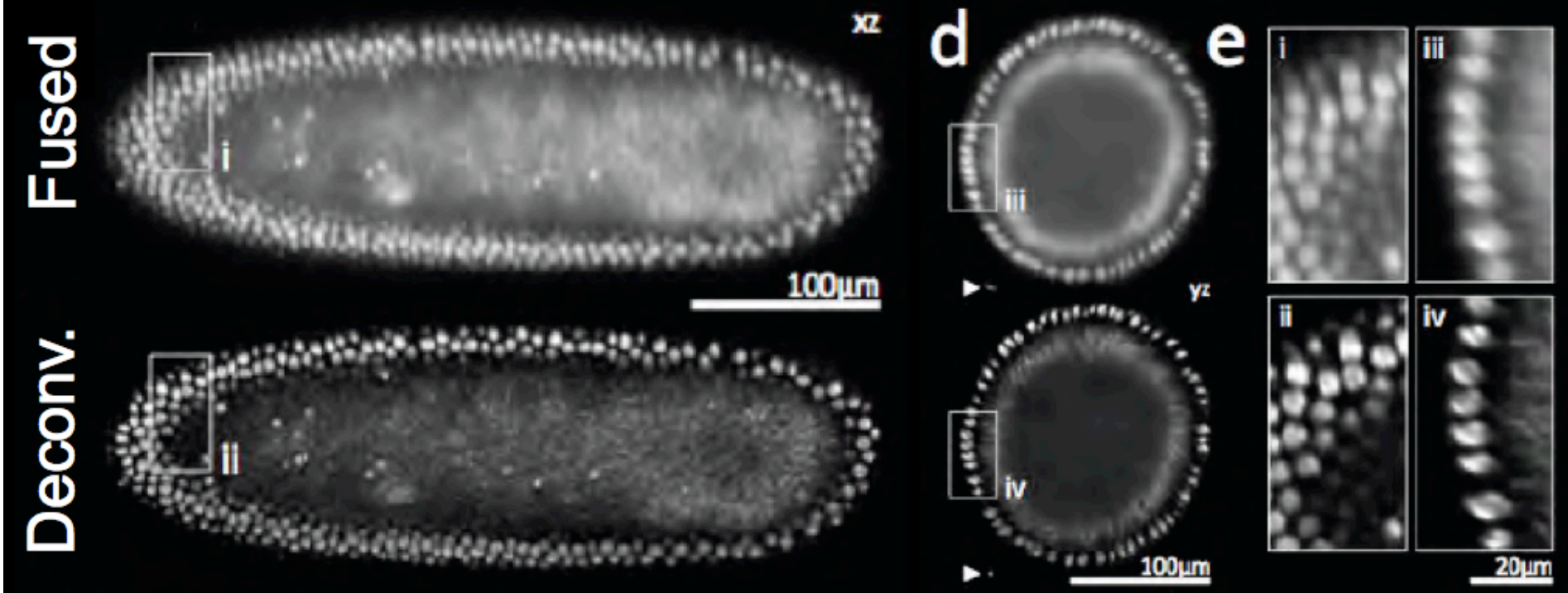
Tassos
Pavlopoulos

Parhyale hawaiensis HisRFP 888 time-points, 3-5 dual sided illumination angles, every 7.5 minutes, acquired on Lightsheet Z1, registered, fused and rendered in Fiji, 5.1 TB raw data


Multi-view Fusion
C. Elegans embryo
4-cell stage
Ph-GFP Lipid binding domain
Angle 0
Angle 60
Angle 120
Angle 180
Angle 240
Angle 300
Content based Fusion
xy
xz
yz

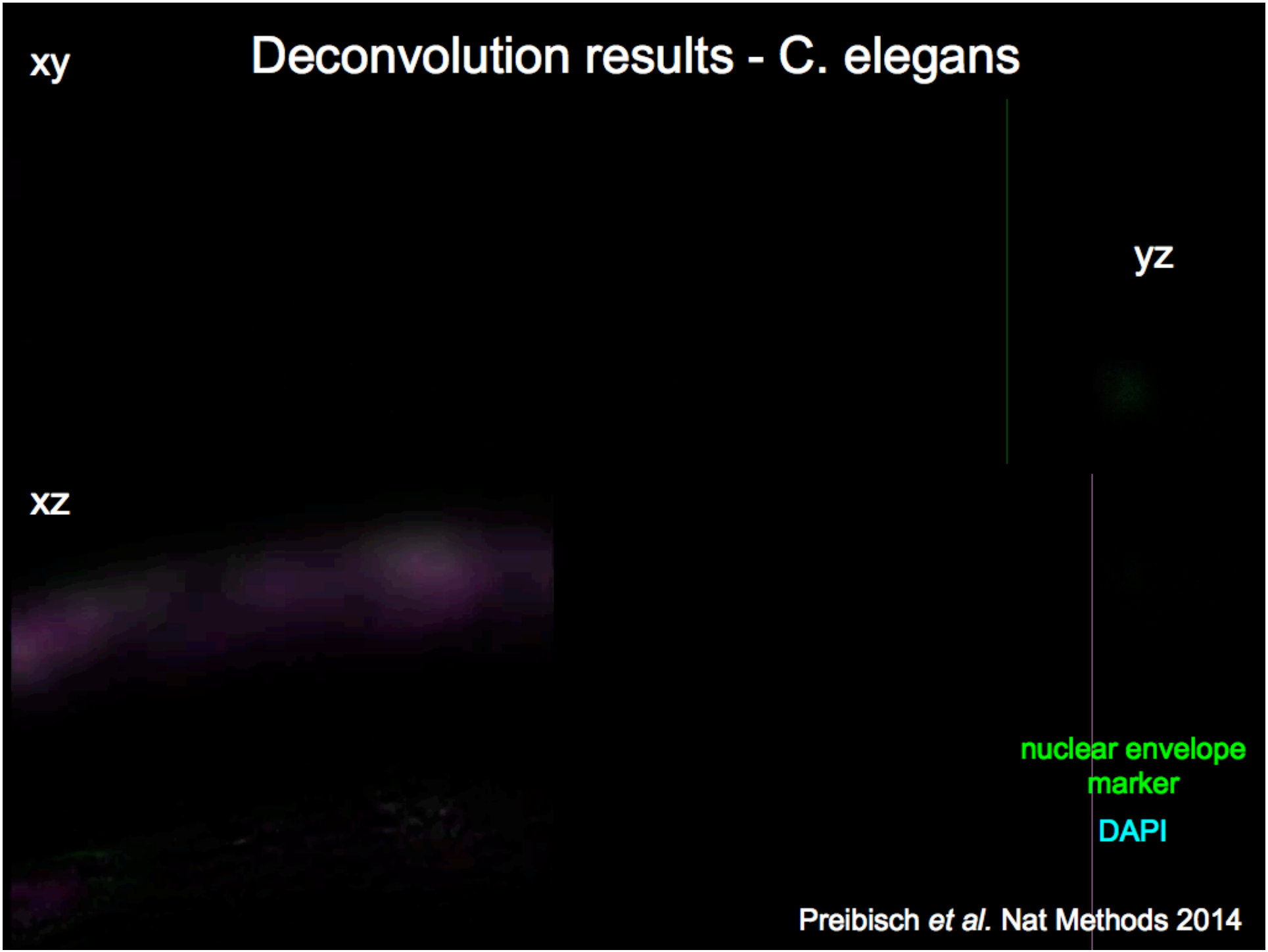

Multi-View Deconvolution
C. Elegans embryo
4-cell stage
Ph-GFP Lipid binding domain
Angle 0
Angle 60
Angle 120
Angle 180
Angle 240
Angle 300
Content based Fusion
Multi-View Deconvolution
xy
xz
yz
xy
xz
yz

Deconvolution results - Drosophila



His-YFP in all cells

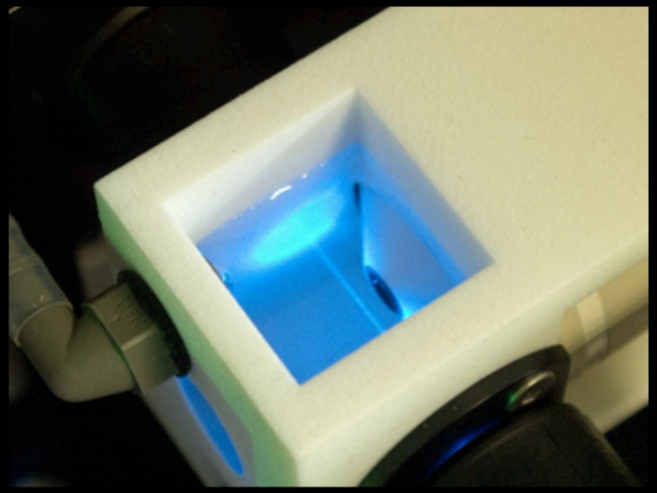
Preibisch *et al.* Nat Methods 2014


xy
Deconvolution results - C. elegans
yz
xz
nuclear envelope marker
DAPI
Preibisch *et al.* Nat Methods 2014

Problems of using SPIM technology:

Data storage

Data Transfer



Data processing

Data Analysis

3D visualization